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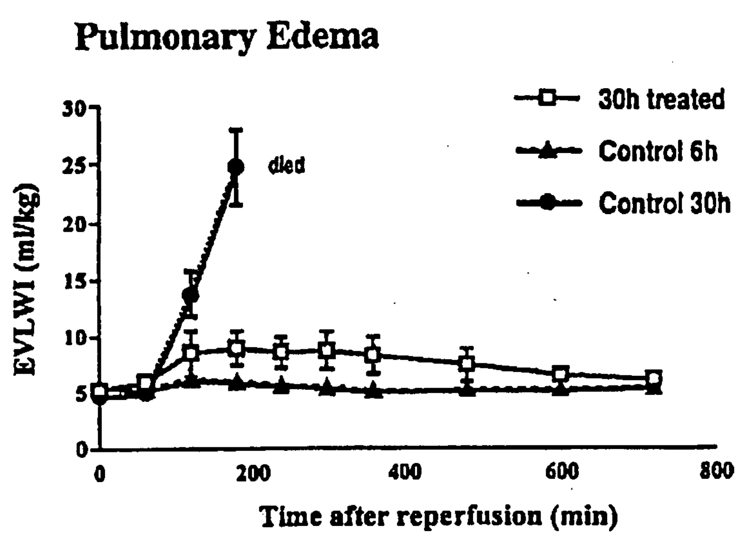
(54) Title: METHOD AND FORMULATION FOR TREATMENT OF VASOCONSTRICTION



(57) Abstract: Compositions and methods useful for achieving therapeutic effects such as the prevention of vasoconstriction and improvement of the preservation and survival of a transplanted organ. More specifically, the compositions exhibit synergy and comprise amounts of two therapeutic agents selected from the group consisting of BH4 and its precursors, on the one hand and membrane permeable cGMP analogues on the other.

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Method and Formulation for Treatment of Vasoconstriction

1. Field of Invention

The invention relates to novel compositions and methods useful for preventing or treating vasoconstriction in organs and tissues, such as in connection with the preservation and reperfusion of transplanted organs in mammals, including man. The novel compositions are synergistic combinations of Tetrahydrobiopterin (BH4) or its precursors together with membrane-permeable analogues of cGMP , such as 8-Br-cGMP, given to the recipient or into the preservation solution sequentially in any order or given simultaneously.

2. Background

2.1 Organ Transplantation

Allotransplantation of whole organs is now an established therapy for end stage organ failure. Routine transplantation of the kidneys, liver, heart, lung, pancreas are performed. Although worldwide more than 60,000 patients each year undergo allotransplantation of one or more organs, the current waiting lists for organ transplantation far exceeds the limited number of organs available. Any measure which extends the donor organ pool by improving the preservation and function of 'marginal quality' organs which may otherwise be rejected would be an important step in reducing mortality & morbidity on the waiting list.

2.2 Organ Procurement

To transplant the organ from a brain dead organ donor, the organ has to be explanted, transported, and again implanted. A certain amount of tissue injury during this procedure is inevitable. To minimize the injury the organ is flushed with ice cold preservation solution to replace the blood and to cool the organ before explantation. The flushing lines are implanted into the main artery of the organ (and portal vein for the flush of the liver) when the circulation of the organ donor is still maintained. At the time of cardiac arrest by the infusion of cardioplegic solution the other organs are flushed through the prepared lines.

After the flush the organs are topically cooled with ice and then explanted. For transport the organs are kept on ice in plastic bags. The maximally tolerated time (ischemic time) between explantation and restoration of the blood flow in the recipient (reperfusion) is four hours for the heart, six hours for the lungs and 24 hours for the liver, the kidneys, and the pancreas.

2.3 Preservation Solutions

The preservation solutions are often specifically designed for each organ. Generally, two types of solutions exist: intracellular solutions with a high potassium content (eg. Euro-Collins solutions for kidneys) and extracellular solutions usually containing colloids as for example dextran (eg. Perfadex for lungs). These solutions often contain additives to improve the preservation of the tissue.

2.4 Ischemia/Reperfusion Injury

Primary non-function in the immediate post-transplant period is a life-threatening complication for all organ recipients with the exception of kidney transplantation, where the organ function can be substituted by dialysis. A number of recent studies indicate that early tissue damage in the transplanted organ also impairs long term function of the graft.

Reperfusion injury is defined as a paradoxical increase in tissue injury at the time of reperfusion that would have not occurred in its absence. Reperfusion of the organ with the recipients blood is a necessary step to reverse the state of ischemia of the organ; the oxygen and energy supply of the organ is restored and toxic metabolites are removed.

Reperfusion, however, may result in activation of a non-specific immune response including hyper-activation of the complement system and polymorphonuclear granulocytes, and the release of pro-inflammatory intermediates.

In addition, this reperfusion response is accelerated by ischemic endothelial damage and endothelium derived pro-inflammatory signals. Endothelial

dysfunction further results in a pathological pattern of eicosanoid release, reduced synthesis of nitric oxide and subsequent vascular dysfunction.

2.5 Strategies to Prevent Ischemia/Reperfusion Injury

The risk of ischemia/reperfusion injury with subsequent primary non-function of the graft can be minimized by short transport- and implantation times and by optimal donor selection. This, however, is not always possible since transport times in the US, for example, are long because of the large size of the country, and, because of the world shortage of donor organs , critically ill patients often have to choose between accepting a "marginal" quality organ or dying on the waiting list..

Therefore, the improvement of organ preservation and protective measures during reperfusion are still top priority objectives in transplantation research. Strategies employed to resolve these problems include: improved preservation solutions, additives to the preservation solutions, and treatment of the recipient during the first hours of reperfusion.

3. Object of Invention

3.1 Nitric Oxide (NO)

Nitric oxide (NO) has been first described in 1987 as a key biological mediator and was named initially endothelium derived relaxing factor (EDRF). Because of its small size, lipophilic nature, and short duration of action (half-life in biological systems 3-30 seconds), NO is an ideal local transcellular messenger. In vascular endothelial cells, NO is synthesized by constitutional NO-synthase (cNOS) from terminal guanidine nitrogen of L-arginine. It diffuses rapidly into subadjacent vascular smooth muscle cells, where it binds to the heme iron complex of soluble guanylate cyclase. The resulting nitrosyl-heme activates guanylate cyclase and stimulates the production of cyclic guanosine 3,5'monophosphate (cGMP), which produces vascular smooth muscle relaxation and vasodilation.

NO, however, has a number of other qualities which are protective for endothelial cells: It modulates neutrophil-endothelial adhesion, it inhibits platelet aggregation,

maintains endothelial barrier properties, and modulates microvascular permeability.

3.2 The NO Pathway During Organ Preservation and Reperfusion

The NO production is impaired during ischemia and reperfusion. This results in vasoconstriction , acceleration of endothelial damage, edema, and subsequent organ dysfunction. Therefore, reinforcement or substitution of the NO-pathway during preservation and reperfusion is crucial for optimal post-transplant organ function. Previously the NO pathway has been substituted by administration of NO-donors as nitroprusside or nitroglycerine in the preservation solution and to the recipient by iv route. In the lung direct inhalation of NO has been evaluated. These techniques have a number of disadvantages and side effects. First the administration of NO-donors as additive to the preservation solution is only effective during the first seconds after initiation of the flush, as the half life of NO is short and as soon as the graft cools down it's effect is blocked. Thus No effect can be expected at the time of reperfusion. Second, administration of NO-donors to the recipient is dangerous, as effective doses of NO-donors induce severe systemic hypotension. Finally, direct inhalation of NO, only possible after lung transplantation, is technically cumbersome and has a potentially high toxic.

The invention described herein allows optimal substitution of the NO-pathway during the entire transplantation process avoiding the toxicity and technical difficulties of the methods described above.

3.3 Tetrahydrobiopterin (BH4)

Tetrahydrobiopterin (BH4) is an endogenous water soluble biopterin with a molecular weight of 314 and is an essential and rate limiting cofactor in the synthesis of nitric oxide (NO) by all known isoforms of nitric oxide synthase (NOS). It is also a natural cofactor for key enzymes in the biosynthesis of several important biogenic signal substances and amines, including epinephrine, norepinephrine, serotonin, and dopamine. (see fig 1) Its deficiency is associated

with a number of disease conditions, including impairment of the NO/cGMP pathway, phenylketonuria as well as some neurological disturbances.

The function of these reactions derives from the ability of BH_4 to react with molecular oxygen to form an active oxygen intermediate that can hydroxylate substrates. In the hydroxylation process, the co-enzyme loses two electrons and is regenerated *in vivo* in an NADH-dependent reaction. Although BH_4 was found to be absolutely essential for nitric oxide synthase activity, the exact function in different forms of the enzyme and the mechanism of action are as yet not clear. .

4. Brief Description of Drawings

Fig. 1: Aromatic Amino Acid Hydroxylases and Function of Tetrahydrobiopterin

Fig. 2: Pulmonary Edema (ExtraVascular Lung Water Index) in transplanted pig lungs as a function of time (mins) after reperfusion. All lungs (except "control 6 h") were subjected to 30 hours of cold ischemia before reperfusion.
(see example 1)

Fig. 3: Gas Exchange (PaO2) in transplanted pig lungs as a function of time after reperfusion. All lungs (except "control 6h") were subjected to 30 hours cold ischemia after reperfusion. (see example 1)

5. Description of the Invention

5.1. Improvement of Posttransplant Graft Function by BH4.

BH4 is the co-enzyme of NO synthases. It inhibits the negative feedback of NO on the NO synthase and therefore improves NO- and subsequently cGMP production. Administration of BH4 during organ (lung) reperfusion (after 20 hours of ischemia) at a dose of 10mg/kg/h over five hours in addition to a bolus of 20 mg/kg 15 minutes before reperfusion resulted in improved graft function and reduced lung edema in a large animal model of lung transplantation.(3, 6).

The major advantage of BH4 over other methods of substitution of the NO-pathway during reperfusion is that NO production is still endogenously regulated

and toxicity and systemic hypotension are avoided. In addition BH4 is easily administrated by iv route and as an endogenous substance is non-toxic.

5.2 Improvement of Organ Preservation by 8-Br-cGMP

8-Br-cGMP is a membrane permeable analogue of cGMP. The rationale for substitution of the NO-pathway during preservation at the level of the second messenger, cGMP, is that during low-temperature organ preservation, all enzymatic reactions leading to synthesis of first (NO) or second messengers are drastically reduced. NO-donors are only effective in the first few seconds after initiation of the flush and thereafter have no effect as the temperature is decreasing in the graft. In addition, due to the short half life of NO, no effect can be expected at the time of reperfusion. This is in contrast to cGMP, the intracellular second messenger of NO, which is still effective when the temperature is decreasing in the graft and which is still present at the time of reperfusion because degrading enzymes do not work during the low-temperature phase. Our own studies on lung transplantation in pigs indicate that membrane-permeable cGMP analogues such as 8-Br-cGMP act as cGMP agonists, reinforcing the cGMP second messenger pathway leading to production of endogenous NO. Thus we have found in the pig lung transplant model that administration of membrane permeable cGMP analogues such as 8-Br-cGMP tend to reduce the degree of reperfusion injury in lung transplantation (4, 5, 6)

5.3 Combined Substitution of the NO pathway by 8-BrcGMP and BH4

The main impact of the described invention is the surprising reinforcement of the NO-pathway during ischemia and reperfusion by combined substitution with 8-Br-cGMP and BH4. The advantages of each substance have been described above.

The lung is the most susceptible organ for ischemia/reperfusion injury due to it's extremely large and fragile endothelial surface. In the large animal model of lung transplantation we could demonstrate that , by using combined treatment with 8-

Br-cGMP (1mg/l) as additive to the flush solution and treatment of the recipient with BH4 over the first five hours after reperfusion (10mg/kg/h), we could successfully transplant lung grafts after a 30 hour preservation time. This is far in excess of the longest lung preservation time previously achieved worldwide in this specific model (which was 24 hours (7)). Since the ischemic injury in the graft increases exponentially woith preservation time (not linearly), these results indicate not only an additive, but a highly surprising synergistic effect of the combined treatment.

The term "synergistic" as used herein means that the effect achieved with the methods and compositions of this invention is significantly greater than the effects that result from methods and compositions comprising the agents of this invention employed separately and in the amounts employed in the methods and compositions hereof.

According to one aspect of this invention, it is thus now possible to achieve a synergistic therapeutic effect in a mammal with amounts of BH4 or its precursor, and a membrane permeable cGMP analogue which, if administered in said amounts singly, are not capable of achieving said effect and which effect is greater than the sum of the effects achieved for each agent separately. Preferred therapeutic effects achieved according to this aspect of the invention are relief of vasoconstriction in a mammal in need thereof and improving preservation and survival of transplanted organs and tissues in a mammal, including man.

The expression "organs and tissues or parts thereof" as used throughout the patent application refers in its widest sense to any type of organ and tissue structure that has been obtained from mammals including man and that preferably can be transplanted to humans and animals by autotransplantation, including muscle, skin or bone flaps, syngeneic transplantation, allotransplantation and xenotransplantation (e.g. from monkey and pig to humans).

Although the detailed etiology behind the intriguing synergy between BH4 and membrane permeable cGMP agonists is not fully clear, it is evident that the two agents synergistically stimulate the generation of endogenous NO from L-

arginine by cNOS, where BH4 acts as a cofactor. It is thus evident that the synergy between BH4 and cGMP agonists will also be of benefit in other therapeutic indications where endogenous NO is known to play a decisive beneficial role.

These include, but are not restricted to , the treatment of conditions caused by undesired smooth muscle contractions, as , for example, in uterine cramps or premature labour where the uterine musculature is triggered by various stimuli to contract at a time when it is undesirable or even life threatening to do so . Another example is the often painful constrictions of urinary bladder musculature associated with the presence of an indwelling urinary catheter.

Vascular smooth muscle spasms are also known to respond to endogenous NO generation, as for example in transient ischemic attacks (TIA), reperfusion injury on declamping a major artery, myocardial infarction, stroke or migraine headaches and a number of neurological disturbances related to diminished NO synthesis in the brain, including ethanol cerebral toxicity.

Other potential applications include treating ocular hypertension associated with glaucoma, hypertensive and atherosclerotic vascular diseases, retinal vein occlusion and ischemic maculopathy. Male impotence has also been shown to respond to stimulation of endogenous NO generation.

The administration of BH4 or precursors and the membrane permeable cGMP analogues can be sequential in time or simultaneous with the sequential method being preferred. For sequential administration, the cGMP analogue can be administered before or after administration of the BH4 but it is preferable to administer the cGMP analogue before the BH4.

Because of the synergistic therapeutic effects achieved by administration of BH4 and/or a cGMP analogue, this invention provides particularly advantageous methods of achieving a therapeutic relief of vasoconstriction or improvement in the preservation and survival of transplanted organs with less than therapeutic levels of BH4 or precursor and/or cGMP membrane permeable analogues. Therefore, in practicing this invention, it is possible to minimize potential

adverse effects which may be associated with larger, therapeutic doses of these agents.

The compositions of this invention comprise an amount of BH4 or precursor; or an amount of membrane permeable cGMP analogue; and a pharmaceutically-acceptable diluent or carrier. The amounts of the BH4 and the cGMP analogue in such compositions are such that each, separately, is not present in an amount sufficient to result in the level of therapeutic effect achieved when combinations of two thereof are administered to a mammal.

A particular advantage of the present invention is that the compositions hereof can comprise amounts of a BH4 or precursor and/or a membrane permeable cGMP analogue which are less that those required for compositions containing only BH4 or a cGMP analogue . Therefore, compositions comprising reduced amounts of BH4 and/or a cGMP analogue according to this invention afford compositions with reduced side effects which may be associated with amounts of the BH4 or cGMP analogue necessary to achieve the same therapeutic effects as the compositions of this invention.

The present invention is not limited in any way to BH4 or to specific BH4 analogues or precursors and/or to specific membrane permeable cGMP analogues but is applicable to all such members of these two groups now known or subsequently discovered or developed. It is the co-administration of BH4 or its analogues or precursors and any membrane permeable cGMP analogue as taught by this invention and not the particular BH4 or cGMP analogue which brings about the synergistic effect of this invention. Nonetheless, a preferred combination for use in the methods and compositions of this invention is tetrahydrobiopterin (BH4) and either 8-br-cGMP or 8-pCPT-cGMP

As discussed above, it is now possible through the practice of this invention to achieve certain desired therapeutic effects using less BH4 and/or less membrane

permeable cGMP analogue than was heretofore possible. The desired therapeutic effects achievable through the practice of this invention include, but are not limited to, relief of vasoconstriction and/or improvement of preservation and survival of a transplanted organ in a mammal. Prior to this invention it was known that a certain amount of BH4 or a certain amount of a cGMP analogue was necessary to achieve desired therapeutic effects. Now, according to this invention, an amount of BH4 less than that necessary to achieve said therapeutic effects can be co-administered with an amount of a membrane permeable cGMP analogue, which amount or amounts are less than that necessary to achieve said therapeutic effects, with the result that synergistic therapeutic effects equal to or greater than said therapeutic effects are achieved.

Further, and significantly, the synergistic therapeutic effects achieved through the use of the methods and compositions of this invention are greater than the sum of the effects achieved through the use of methods and compositions employing either BH4 or a cGMP analogue alone in amounts equal to the amounts used in the methods and compositions herein.

In practicing the methods of this invention, which comprise administering, simultaneously or sequentially and in any order, BH4 and a membrane permeable cGMP analogue, such administration can be orally, bucally, parenterally, by inhalation spray, rectally or topically. It is preferred that such administration be parenterally. It is even more preferred that such administration be parenterally and sequentially. The term "parenterally" as used herein includes, but is not limited to, subcutaneous, intravenous, intramuscular and intrasternal injections and infusion techniques. When the BH4 and/or the cGMP analogue are administered sequentially, the administration of each can be by the same method or by different methods.

The pharmaceutical compositions of this invention include compositions which comprise either BH4, its analogues and precursors or a membrane permeable cGMP analogue in an amount less than that necessary to achieve the desired therapeutic effect together with a pharmaceutically-acceptable diluent or carrier

and compositions which comprise two of the agents, each of which is present in an amount which is less than that necessary to achieve the desired therapeutic effect alone, together with a pharmaceutically-acceptable diluent or carrier.

The compounds of this invention can be orally administered in a wide variety of different dosage forms, i.e., they may be formulated with various pharmaceutically acceptable inert carriers in the form of tablets, capsules, lozenges, troches, hard candies, powders, sprays, aqueous suspensions, elixirs, syrups and the like. Such carriers include solid diluents or fillers, sterile aqueous media and various non-toxic organic solvents, etc. Moreover, such oral pharmaceutical formulations can be suitably sweetened and/or flavored by means of various agents of the type commonly employed for such purposes.
In general, the compounds of this invention are present in such oral dosage forms at concentration levels ranging from about 0.5% to about 90% by weight of the total composition, in amounts which are sufficient to provide the desired unit dosages. Other suitable dosage forms for the compounds of this invention include, but are not limited to, controlled release formulations and devices well known to those who practice in the art.

For purposes of parenteral administration, solutions of the compounds in biodegradable oils or in aqueous propylene glycol may be employed, as well as sterile aqueous solutions of the corresponding pharmaceutically-acceptable salts. Such aqueous solutions should be suitably buffered if necessary, and the liquid diluent rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular and subcutaneous injection purposes. In this connection, the sterile aqueous media employed are readily obtained by standard techniques well known to those skilled in the art. For instance, distilled water is ordinarily used as the liquid diluent and the final preparation is passed through a suitable bacterial filter The necessary steps should be taken throughout the preparation of these injectable solutions to insure that the final products are obtained in a sterile condition.

For purposes of transdermal administration, the dosage form of the particular compound or compounds may include, by way of example, solutions, lotions, ointments, creams, gels, suppositories, rate-limiting sustained release formulations and devices therefor. Such dosage forms comprise the particular compound or compounds and may include ethanol, water, penetration enhancer and inert carriers such as gel-producing materials, mineral oil, emulsifying agents, benzyl alcohol and the like. Specific transdermal flux enhancing compositions are disclosed in European Patent Application 271,983 and European Patent Application 331,382 , the teachings of which are incorporated herein by reference.

The dosage of the BH4 and/or membrane permeable cGMP analogue necessary to achieve the desired therapeutic effect is within the skill of those who practice in the art having the benefit of the disclosure herein. Dosage ranges for BH4 have been reported with representative dosages being 10 – 20 mg/kg / hr I.V. and for 8-br-cGMP, a cGMP analogue, 1 mg/ kg has typically been used in the organ preservation fluid. The dosages to be employed according to this invention may be varied depending upon the requirements of the patient, the severity of the condition being treated and the compounds being administered. Further, the daily dosages to be administered may be divided and administered in portions during the day. The dosage or dosages which will result in optimal synergistic effects is achieved by coordinating the pharmacokinetic properties, such as volume of distribution and Tmax, of the therapeutic agents of this invention so that the therapeutic windows of each agent overlap to the maximum extent possible. Such dosages are readily determined by one skilled in the art enabled by the disclosure herein.

The methods herein disclosed are useful for preventing (if given prior to the onset of symptoms) or reversing acute pulmonary vasoconstriction, such as may result from pneumonia, traumatic injury, aspiration or inhalation injury, fat embolism in the lung, acidosis, inflammation of the lung, adult respiratory distress syndrome, acute pulmonary edema, acute mountain sickness, asthma, post cardiac surgery

acute pulmonary hypertension, lung transplantation, persistent pulmonary hypertension of the newborn, perinatal aspiration syndrome, hyaline membrane disease, acute pulmonary thromboembolism, heparin-protamine reactions, sepsis, status asthmaticus, or hypoxia (including that which may occur during one-lung anesthesia), as well as those cases of chronic pulmonary vasoconstriction which have a reversible component, such as may result from chronic pulmonary hypertension, bronchopulmonary dysplasia, chronic pulmonary thromboembolism, idiopathic or primary pulmonary hypertension, or chronic hypoxia.

EXAMPLE

The example presented below and figs 2 & 3 illustrates how the synergistic effects of combined therapy with BH4 and a membrane permeable cGMP analogue and agonist, 8-Br-cGMP, permit a dramatic improvement in the long term preservation of the pig lung. Previous attempts to extend the safe preservation time for cold transport of lungs from the donor to the recipient have been hampered by the development of irreversible "ischemia – reperfusion injury" to the lung tissue such that the maximum cold storage time for pig lung under the exacting conditions of this specific experimental pig model has previously not exceeded 24 hours.(7)

Since the degree of "ischemia-reperfusion" injury to lung tissue (i.e. the injury largely caused by free radicals both during ischemia and on reperfusion of the transplant by the recipients blood) increases exponentially with ischemic time, an extension of preservation time from 24 hrs to 30 hrs represents a major challenge to the transplant surgeon since the additional injury involved is substantially more than the 25% extension in storage time. Despite this tough model, all lungs preserved for 30 hours in a solution containing 1 mg/kg 8-Br-cGMP and thereafter reperfused for 5 hrs with BH4 survived with good functional recovery whereas none of the control lungs which had been preserved for 30 hrs without the addition of both 8-Br-cGMP and BH4 survived. (see figs 2 & 3)

Previously published work from the same team working with the same pig model under the same conditions showed that the corresponding doses of BH4 and 8-Br-cGMP used separately could maintain safe lung preservation up to 20 hrs. (3, 4, 5, 6) The remarkable results with the combination of BH4 and 8.Br-cGMP was a very surprising achievement and clearly indicates a synergistic effect of, as yet, unknown etiology.

EXAMPLE 1

Successful lung transplantation after 30 hours preservation with Perfadex and combined use of BH4 and 8-Br-cGMP.

Background: Substitution of the NO-pathway improves early graft function following lung transplantation. This was shown in separate experiments by our group with 8-Br-cGMP (second messenger of NO) singly in the flush solution and Tetrahydrobiopterin (BH4, coenzyme of NO synthase) singly after reperfusion.(3, 5, 6). In the present study the combined treatment with 8-Br-cGMP and BH4 was evaluated.

Methods: Unilateral left lung transplantation was performed in weight matched outbred pigs (24-31 kg). Grafts were preserved for 30 hours(n=5) . 8-Br-cGMP (1 mg/kg) was added to the flush solution (LPD, 1.5L, 1 C) and BH4 (10mg/kg/hr) was given to the recipient for 5 hrs after reperfusion. For control reasons lungs were transplanted after 6 hrs and 30 hrs preservation time respectively (n=2) and PGE1 (250ug) was given into the pulmonary artery (PA) prior to flush. In all recipients one hr after reperfusion the contralateral right PA and bronchus were ligated to assess graft function in the transplanted lung only. Extravascular lung water index (EVLWI), hemodynamic variables, and gas exchange (PaO2) were assessed during a 12 hr observation period after the 30 hours of ischemia. Lipid peroxidation (TBARS) and neutrophil migration to the allograft (MPO activity) were measured at the end of the assessment.

Results: All recipients with combined NO substitution survived the 12 hr assessment after 30 hr graft preservation with good pulmonary function. None of

the untreated animals with grafts preserved for 30 hrs survived more than 4 hrs after reperfusion.

Conclusion: Our data indicate that the combined substitution of the NO pathway during preservation and reperfusion reduces ischemia/reperfusion injury dramatically. This treatment allows safe prolongation of the preservation time to 30 hrs in this model.

See figs 2 and 3 attached.

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CLAIMS

1. A first pharmaceutical composition for use with a second pharmaceutical composition for achieving a therapeutic effect comprising the prevention or treatment of vasoconstriction or improvement of preservation and survival of transplanted organs in a mammal in need thereof, which effect is greater than the sum of the therapeutic effects achieved by said first and second pharmaceutical composition separately and which second pharmaceutical composition comprises an amount of tetrahydrobiopterin (BH4) or its precursor, and said first pharmaceutical composition comprising a synergistic effective amount of a membrane permeable cGMP analogue and a pharmaceutically acceptable diluent or carrier.

2. A pharmaceutical composition for achieving a therapeutic effect comprising prevention or treatment of vasoconstriction or improvement of preservation and survival of a transplanted organ in a mammal in need thereof which composition comprises synergistic effective amounts of:
(a) BH4 or its precursor and
(b) a membrane permeable cGMP analogue,
wherein the amount of (a) alone and the amount of (b) alone is insufficient to achieve the therapeutic effect; and wherein the combined effect of the amounts of (a) and (b) is greater than the sum of the individual therapeutic effects achievable with the amounts of (a) and (b); and a pharmaceutically acceptable diluent or carrier.

3. The pharmaceutical composition according to claim 2 wherein (a) is BH4 and (b) is 8-Br-cGMP or 8-pCPT-cGMP or dibutyryl cGMP.

4. The pharmaceutical composition according to claim 3 wherein (b) is 8-Br-cGMP..

5. A pharmaceutical composition for achieving a therapeutic effect comprising preventing or treating vasoconstriction or improving the preservation and survival of transplanted organs in a mammal in need thereof which composition comprises synergistic effective amounts of: (a) BH4 and (b) a membrane permeable cGMP analogue wherein the amount of BH4 alone and the amount of cGMP analogue alone is insufficient to achieve the therapeutic effect; and wherein the combined effect of the amounts of BH4 and a membrane permeable cGMP analogue is greater than the sum of the individual therapeutic effects achievable with the amounts of BH4 and a membrane permeable cGMP analogue., and a pharmaceutically acceptable diluent or carrier.

6. A method for achieving a synergistic therapeutic effect comprising prevention or treatment of vasoconstriction or improving the preservation and survival of transplanted organs in a mammal in need thereof which method comprises administering to said mammal synergistic effective amounts of (a) BH4 and (b) 8-Br-cGMP or 8-pCPT-cGMP or dibutyryl cGMP wherein the amount of BH4 alone and the amount of 8-Br-cGMP or 8-pCPT-cGMP or dibutyryl cGMP alone is insufficient to achieve the therapeutic effect; and wherein the combined effects of the amounts of BH4 and 8-Br-cGMP or 8-pCPT-cGMP or dibutyryl cGMP administered is greater than the sum of the individual therapeutic effects of the amounts of BH4 and 8-Br-cGMP or 8-pCPT-cGMP or dibutyryl cGMP administered.

7 . The method according to claim 6 wherein an amount of BH4 and 8-Br-cGMP or 8-pCPT-cGMP or dibutyryl cGMP are administered simultaneously.

8.. The method according to claim 6 wherein an amount of 8-Br-cGMP or 8-pCPT-cGMP or dibutyryl cGMP is administered before an amount of BH4

9. The method according to claim 6 wherein the therapeutic effect comprises improving the preservation of an organ awaiting transplantation and improving the survival of a transplanted organ.

10. The method according to claim 6 wherein the therapeutic effect comprises prevention or treatment of conditions and diseases characterised by vasoconstriction , including hypertension, vascular spasm, as in cerebral hemorrhage, stroke, TIA and infarction, vascular reperfusion injury after declamping an artery.

11. The method according to claim 6 where the therapeutic effect comprises treatment of diseases related to a deficiency in endogenous NO generation , as in male impotence, artherosclerosis,

12. The method according to claim 6 where the therapeutic effect comprises treatment of diseases or conditions caused by excessive, pathological or undesirable smooth muscle contractions, or spasm, as in uterine spasm, some forms of premature labour, ocular hypertension in glaucoma, transient ischemic attacks (TIA), stroke, male impotence.

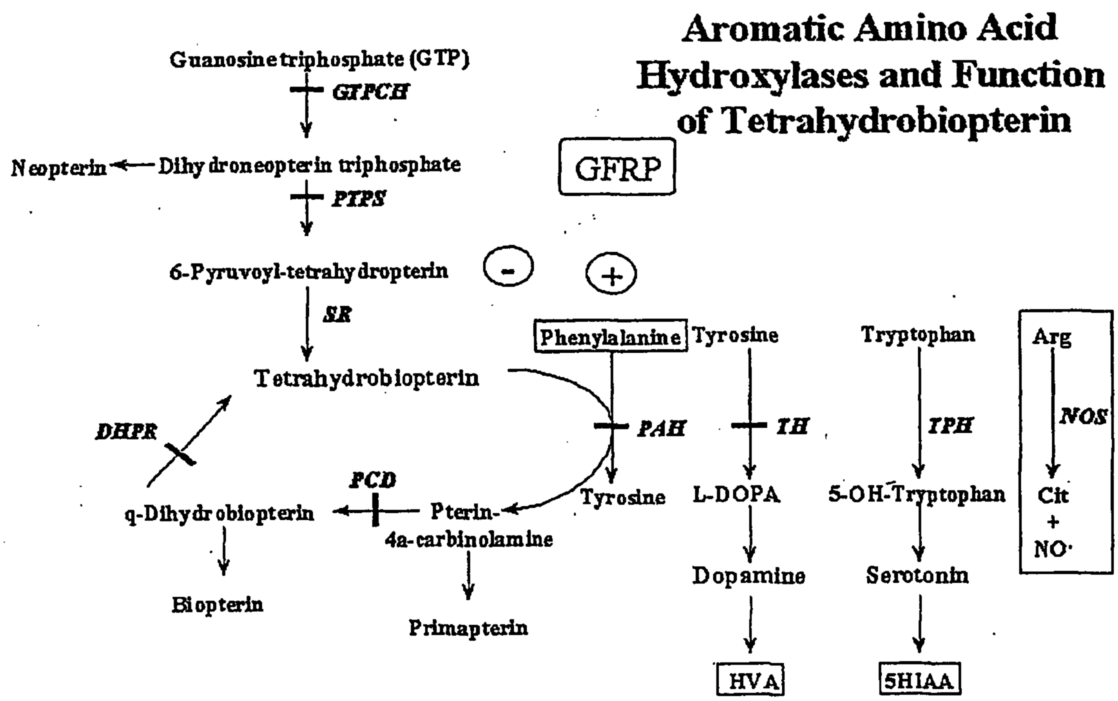
Aromatic Amino Acid Hydroxylases and Function of Tetrahydrobiopterin
Guanosinetriphosphate (GTP)
GTPCH
Neopterin
Dihydroneopterin triphosphate
PTPS
6-Pyruvoyl-tetrahydropterin
SR
Tetrahydrobiopterin
DHPR
q-Dihydrobiopterin
PCD
Pterin-4a-carbinolamine
Biopterin
Primapterin
GFRP
-
+
Phenylalanine
PAH
Tyrosine
Tyrosine
TH
L-DOPA
Dopamine
HVA
Tryptophan
TPH
5-OH-Tryptophan
Serotonin
5HIAA
Arg
NOS
Cit
+
NO·

Fig. 1

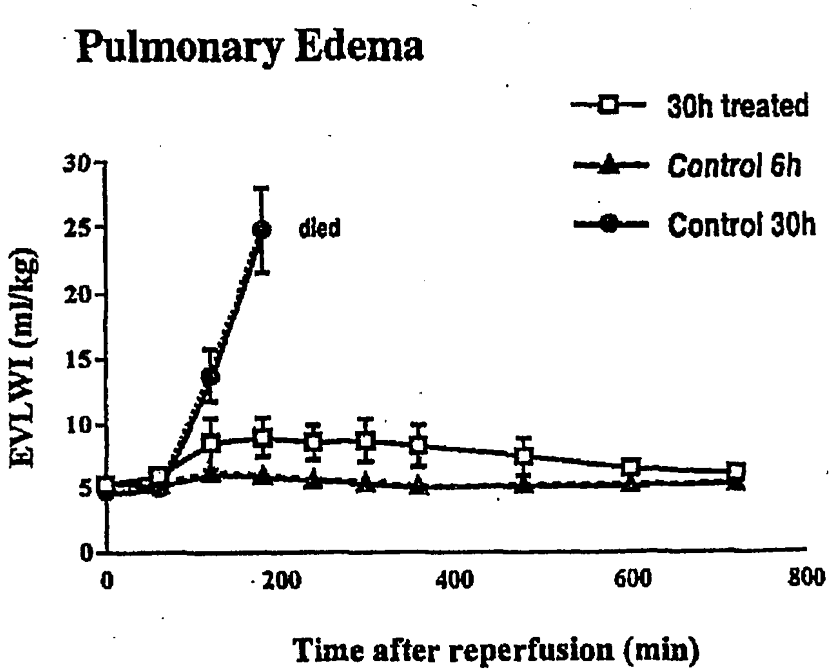
Pulmonary Edema
30h treated
Control 6h
Control 30h
died
EVLWI (ml/kg)
0
5
10
15
20
25
30
0
200
400
600
800
Time after reperfusion (min)

Fig. 2

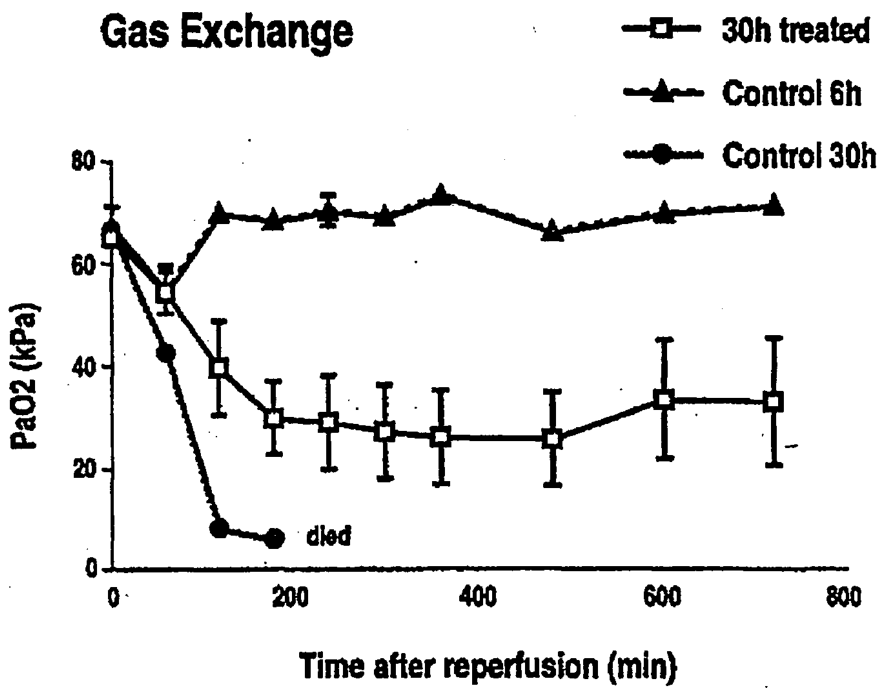
Gas Exchange
30h treated
Control 6h
Control 30h
80
60
40
20
0
PaO2 (kPa)
died
0
200
400
600
800
Time after reperfusion (min)

Fig. 3